chemosensory cilia of Caenorhabditis elegans two kinesin-2-family motors, kinesin-II and OSM-3-kinesin, cooperate to build and maintain the cilium, in a process called intraflagellar transport (IFT). In order to quantitatively asses IFT-kinesin function at endogenous expression levels, we have generated transgenic worms using Mos1-mediated single-copy integration of transgenes encoding fluorescently-labeled-IFT kinesins. Ultrasensitive wide-field and confocal fluorescence microscopy allows accurate mapping, counting, tracking and correlation of these molecular machines inside living, multicellular organisms. This approach allows unprecedented insight into IFT and motor-driven processes in general.

**1920-Pos Board B690**

**Coarse-Grained Model of Cooperative Chloroplast Transport in Moss**

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Organelle motility is essential for the functioning of the eukaryotic cell. Actively modifying intracellular structures allows cells to change and adapt to different conditions. One of these cellular structures is the microtubule cytoskeleton, which is comprised of polarized filaments that function as tracks to transport cargo via molecular motors. Recent studies have revealed the importance of cooperative transport in living cells. In the moss Physcomitrella patens, reorganization of the chloroplasts is critical to adapt to changes in light quality and intensity. In this work, we performed detailed analysis of the transport of chloroplasts in protonemal moss cells, and showed that their transport is facilitated by molecular motors and the microtubule cytoskeleton. Our findings were recapitulated using coarse-grained modeling of this cargo transport over different microtubule network topologies. Our simulations include a detailed description of motor and microtubule dynamics, in the presence of thermal fluctuations. Our modeling sheds light into the molecular mechanisms at play in the photo-relocation response of chloroplasts in moss.

**1921-Pos Board B691**

**Logistics of Intracellular Transport Required for Cell Wall Assembly**

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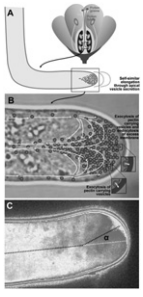
In plants, cellular growth requires the assembly of extensive amounts of new cell wall surface. The targeted deposition of building material through exocytosis - cell wall polymers, enzymes and membrane material - is therefore a crucial regulatory feature in plant development. The spatial and temporal regulation of the delivery of cargo vesicles to the target surfaces are poorly understood. Spatio-temporal image correlation spectroscopy (STICS) was used to quantify the intracellular dynamics of secretory vesicles and of the actin arrays in pollen tubes - rapidly and polarly growing plant cells. The dynamic profiles were used to validate mathematical models for vesicular trafficking. Boundary conditions were the expanding cell wall (Fig.1A) and the actin array whose shape was obtained by imposing a steady state and constant polymerization rate of the actin filaments (Fig.1B). The model correctly predicted the vesicle flow patterns in different types of pollen tubes and provides an explanation for flow dynamics in cellular regions devoid of actin cytoskeleton. It will serve as a basis for understanding how pollen tubes are able to regulate their morphogenetic pattern, for example when responding to a directional trigger by changing the growth direction (Fig.1C).




**1922-Pos Board B692**

**Understanding Non Brownian Dynamics of Intracellular Transport**

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Understanding insulin granule transport in live beta cells is a complicated task. Traditionally, the diffusion coefficients and the velocity of insulin granules measured via particle tracking techniques are used to characterize the dynamics, which requires the assumption that the dynamics to be either purely diffusive or ballistic. This is not the case for insulin granules. We use a variety of statistical data analysis, to show that insulin granule vesicles in their pathway, which leads to exocytosis performs a subordinated intercellular transport mechanisms, which leads to a statistical anomalous dynamics.

**1923-Pos Board B693**

**Regulating Intracellular Transport with External Stimuli**

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Cellular physiology and metabolism depend on mechanisms that regulate the transport macromolecules and organelles along cytoskeletal filaments. In many cases, these cargos are transported by multiple motor proteins that work either agonistically or antagonistically to control the directions of cargo motion. A number of new experimental techniques have been developed to study how the interactions between motors affect cargo transport, examining these problems inside of living cells has been much more challenging. The main aim of this study is to develop an intracellular assay that facilitates examination of mechanism governing cargo transport by multiple motors. To do so, we have designed cell lines that allow the coupling an uncoupling of motors to and from cargos via the application of an external stimuli. These cells provided control over the number of cargo attachment sites and the number of motors that are attached to these sites. The use of this system to examine the cooperation and competition of motors will be discussed.

**1924-Pos Board B694**

**Mapping Vesicle Trafficking during Plant Cell Cytokinesis using Spatio-Temporal Image Correlation Spectroscopy**

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The delivery of new cell wall material to the forming cell plate of a dividing plant cell requires intricate coordination of secretory vesicle trafficking. The vesicles need to be transported rapidly and efficiently to precise locations in the cell at specific times in order for cell division to occur normally. The trafficking of vesicles is mediated by the cytoskeleton via complex regulatory mechanisms. However, the dynamics of the vesicle delivery is difficult to measure in living cells due to their small size and high density. The vesicle dynamics are measureable via Spatio-Temporal Image Correlation Spectroscopy (STICS), a fluorescence fluctuation method that was initially developed to measure the directed transport or flow of proteins inside living cells. STICS relies on calculating the complete space-time correlation function of the intensity fluctuations between images of a time series obtained using a fluorescence microscope. Here, we use STICS to analyze laser scanning confocal microscopy image time series to obtain quantitative information on secretory vesicle dynamics in plant cells between their production from Golgi stacks and the final step of vesicle docking and fusion at the cell plate initiation site. We were able to map the direction and magnitude of vesicle movement at the different stages of cell division. This allowed us to determine the range of velocities of vesicles and to observe the varying flow patterns and the fast changing nature of their dynamics during the formation of the cell division plate.

**1925-Pos Board B695**

**Intracellular Trafficking of Lipid Gene Vectors Investigated by Three-Dimensional Single Particle Tracking**

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Three-dimensional single particle tracking (SPT) was applied to investigate the intracellular trafficking of multicomponent (MC) lipoplexes in CHO-K1 cells. In untreated (NT) cells, we have found that: (i) intracellular lipoplex motion was either directed or Brownian;(ii) the occurrence of directed motion was more frequent (more than 70%) than the Brownian one;(iii) within experimental error, the Brownian motion (D~0.7•10-3 um$\perp$2/s) was faster than the directional movement (D~0.35•10-3 um$\perp$2/s);(iv) the directed motion mean velocity was about v=0.032 um/s;(v) the calculated three-dimensional asphericity, A3, was close to unity denoting the privileged occurrence of movement along a direction. To elucidate the role of the cytoskeleton structure in the lipoplex trafficking, cells were treated with cytoskeleton (actin microfilaments and microtubules) polymerization inhibitors (Latrunculin B and Nocodazole, respectively). In inhibitor-treated cells, we have found that: (i) the percentage of directional movement decreased balanced by the simultaneous increase in the occurrence of Brownian motion;(ii) reduction of directional movement was large but never complete. Such observation might reflect either an incomplete disruption of cytoskeleton network by drug treatment and/or its recovery due to the kinetic profile of the drugs employed;(iii) the effect of Nocodazole on the reduction of directional movement was definitely stronger than that of

Latrunculin B;(iv) lipoplex mobility increased. Indeed, within each motion category (i.e. directed or Brownian), the diffusion coefficients were, in general, higher than the corresponding values obtained in NT cells. However, a very precise trend could not be found probably due to the low accuracy of experimental data;(v) within experimental error, the mean velocities were in the same range of those obtained in NT cells;(vi) the calculated asphericities were lower than that calculated in NT cells and were found to be close to the theoretical random walk value.

**1926-Pos Board B696**
**Mitochondria - A Potential Roadblock for Axonal Transport**
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Axonal transport of materials in neurons is an important process that directly affects the survival of neurons. Moreover, defects in this process have been linked to various neurodegenerative diseases. This work examines the interaction between mitochondria and cargoes transported along axon of dorsal root ganglion neurons using two-color imaging on a pseudo-TIRF setup. The result shows that cargoes transported along axon are more likely to slow down and pause in the vicinity of mitochondria. This propensity increases when drug is used to induce mitochondrial swelling. This can be explained as the large size of mitochondria (relative to the thin shaft of axon) can potentially inhibit cargo transport in axon through steric hindrance. Moreover, this finding also indicates that swollen mitochondria observed in some neurogenerative diseases may be one of the causes for axonal transport failure.

**1927-Pos Board B697**
**Correlative Real-Time and Super Resolution Imaging of Mitochondrial Dynamics**
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Mitochondria are energy producing organelles that play essential functions in all aspects of cell biology. In most mammalian cells mitochondria exist in the form of a highly dynamic interconnected network. Live cell imaging experiments revealed that this network undergoes dramatic shape re-organization as motor proteins actively pull the mitochondrial membrane. In addition, mitochondria that exist as individual organelles can be actively transported from one location to another. Mitochondrial shape changes and their active transport involve microtubules and play key roles in mitochondrial function However, several questions remain unanswered regarding the dynamic behavior of mitochondria: how often do mitochondria change tracks as they are transported along microtubules, how do the underlying microtubule tracks influence the observed shape changes and can mitochondrial membrane be pulled along multiple microtubule tracks.
In order to relate the dynamic events of the mitochondrial network to the underlying cytoskeleton, we developed a sequential live-cell and super-resolution imaging approach. Live cell imaging allowed us to track the movement of mitochondria and characterize their dynamic behavior. We could stop the dynamics at a specific time point by *in situ* fixation on the microscope stage. Subsequent immuno-staining followed by super-resolution imaging using Stochastic Optical Reconstruction Microscopy (STORM) allowed us to obtain a high resolution (~20 nm) image of the underlying microtubule network. The microtubule network was stabilized through treatment with low concentrations of drugs (nocodazole and taxol) and did not change appreciably during live cell imaging. As a result, we could overlay the dynamic behavior of mitochondria with the high resolution images of microtubule tracks by using fiduciary markers for alignment. This approach allowed us to correlate mitochondrial dynamics and the microtubule tracks at high resolution in order to answer the questions that we pose above.

**1928-Pos Board B698**
**Intraflagellar Transport Powers Flagellar Surface Motility in Chlamydomonas**
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Chlamydomonas reinhardtii is a unicellular biflagellate alga that exhibits whole-cell gliding motility and directed transport of flagellar membrane glycoproteins (FMG) on the flagellar membrane. There are indications that gliding motility and FMG movement are manifestations of intraflagellar transport (IFT), in which kinesin-2 and dynein-1b move large arrays of proteinaceous particles to and from the distal end of the flagellum. It is of much interest to determine whether IFT plays a role in dynamic flagellar turnover and whole cell motility. We have studied all three types of motility in live Chlamydomonas cells by a combination of advanced single-molecule fluorescence and force microscopy techniques. We have observed that FMGs rapidly associate and dissociate from IFT cargos and are transported by IFT machinery back and forth along the flagella. Individual retrograde IFT trains transiently pause, presumably due to adhesion of FMGs to a glass surface. Forces generated by dynein-1b motors attached to the paused IFT trains pull the whole cell in the opposite direction relative to the substrate. A single IFT train is transported by at least 4–5 motors on average in each direction, and opposite polarity motors do not interfere with each other along the length of the flagellum. The results have suggested that IFT is a highly regulated bidirectional transport, which also generates force for flagellar surface motility in Chlamydomonas.

**1929-Pos Board B699**
**Axonal Traffic Control in Live Neurons by Tailor-Designed Magnetic Forces**
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The axon acts as a conduit for organized transport of material, between the cell body and the synapse, which is essential for the function and survival of neurons. Axonal traffic jams caused by local accumulation of cargo have been implicated in many neurodegenerative diseases. In order to study the neuronal response to axonal traffic jams we need new noninvasive assays capable of A) slowing/stalling axonal cargo by external forces to induce controlled traffic jams B) monitoring the perturbed transport and the ensuing neuronal response in real time. Here, we present an integrated methodology based on microfluidic neuron culture, high-gradient magnetic trapping and multi-color TIRF imaging that permits external control of axonal traffic in live neurons via magnetic forces. We fabricated a novel microfluidic device for neuron culture by patterned electrodeposition of soft micromagnets (permalloy) on glass coverslips. In the presence of an external magnetizing field, the soft micromagnetic pattern gives rise to local zones of high magnetic gradients. By culturing neurons in this device, with axons aligned along these high gradient zones, we can exert pN forces on axonal endosomes carrying magnetic nanoparticles (MNPs, 50 nm). The magnetic forces counter the molecular motor forces to physically stall the endosomes, which leads to axonal traffic jams. The axonal growth and the delivery of MNP-loaded axonal endosomes along the high gradient zones are achieved by microfluidic compartmentalization of neuron culture. We have successfully A) compartmentalized DRG neurons in prototype magnetic devices B) characterized lectin-mediated axonal transport of 50 nm MNPs by pseudo-TIRF imaging, with/without external magnetic forces C) demonstrated the magnetic induction of controlled axonal traffic jams. These advances can potentially unravel the cooperative mechanics of multi-motor axonal transport and also elucidate the generic links between traffic jams, axonal swellings and neurodegeneration.

**1930-Pos Board B700**
**Study of BDNF Transcytosis in Hippocampal Neurons**
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Brain derived neurotrophic factor (BDNF), a member of the neurotrophin family, plays important roles in neuron survival, development and synaptic efficacy. It is believed that exogenous BDNF binding to TrkB activates three major signaling pathways: MAPK, PI3K and PLC-gamma. One important issue under these regulation mechanisms is whether and how BDNF is transported into neurons and its intracellular translocation. Furthermore, whether such exogenous BDNF is released and taken up by another neuron and involved in neuron to neuron communication has not been studied. In this work, BDNF conjugated to quantum dot is traced after it is taken by hippocampal neurons. A compartmentalized microfluidic device has been designed to separate axons and dendrites from each other. By applying Qdot-BDNF only to the axon chamber and observing some Qdot-BDNF leaving from the dendrite chamber, it is clearly proved that BDNF up-taken from the axon terminal can be translocated all the way to dendrites. Qdot-BDNF entering and leaving the soma has also been directly observed. Whether BDNF is released from the axon or dendrite terminal is under investigation. It is hoped that at the end of this work, an overall picture of the whole regulation cycle of exogenous BDNF and whether its role as a chemical communicator through the neuronal network can be clearly shown.